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Effects of betahistine on the spatiotemporal response properties of vestibulospinal neurons to labyrinthine volleys

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Abstract

Betahistine, a drug used in the treatment of vestibular disorders, speeds-up the recovery from hemilabyrinthectomy in experimental animals, likely through the activation of histamine receptors. In order to better understand the mechanism of action of this drug we investigated, in adult, urethane anesthetized rats, whether betahistine modifies the spatial (directional) and temporal response properties of vestibular nuclear neurons to the labyrinthine input, as well as the convergence of different labyrinthine signals on single units. Extracellular single-unit activity was recorded from the caudal, spinal-projecting region of the vestibular nuclei during tilt of the animal, before and after i.p. injection of betahistine. The two orthogonal directions of maximal and minimal response to tilt, as well as the corresponding gains were determined for each neuron. Betahistine reduced the maximal response gain of units showing larger basal values of this parameter and increased it in neurons with smaller basal values, while the minimal response gain was on the average raised. These changes led to a significant decrease in the spatial specificity of the neurons, suggesting that betahistine affects the process of spatiotemporal convergence on vestibular units, likely through a rearrangement of the various inputs. This could be related to the effect of the drug on vestibular compensation.

Keywords: Betahistidine; Vestibular disorder; Electrophysiology

1. Introduction

Betahistine is a histaminergic drug with a week H_1 agonist (Hill et al., 1978; Young et al., 1993; Arrang et al., 1985) and a strong H_2 antagonist action (Arrang, 1983; Arrang et al., 1985), widely used in the treatment of Meniere's disease and in other syndromes of peripheral vertigo (Rascol et al., 1995; see Mira, 2001 for ref.). It has been recently shown that high doses of this molecule may speed up the process of vestibular compensation in an experimental animal model (Lacour and Sterkers, 2001). The mechanism of action of betahistine it is not known with certainty, but the drug may affect the cochlear (Suga and Snow, 1969; Martinez, 1972; Laurikainen et al., 1993, 1994, 1998) and vestibular blood flow (Dziadziola et al., 1999). Moreover, since betahistine may cross the blood–brain barrier (Arrang et al., 1985), it may interact with histamine H_1 and H_3 receptors localized within the central nervous system and, in particular, at the level of the vestibular nuclei (Serafin et al., 1993; Yabe et al., 1993; Tighilet and Lacour, 1996; Lacour, 1998; Pillot et al., 2002), that receive a heavy histaminergic input from the hypothalamus (Tighilet and Lacour, 1996; Lacour, 1998). It is known that betahistine reduces, in vivo, the spontaneous discharge and the response to horizontal rotation of neurons located in the medial vestibular nucleus (Kawabata et al., 1991) as well as the polysynaptic responses of lateral vestibular nucleus neurons to labyrinthine stimulation (Unemoto et al., 1982). In slice preparation betahistine exerts a weak excitatory effect on the activity of vestibular neurons, but decreases their response to histamine (Wang and Dutia, 1995). At peripheral level, it decreases the spontaneous discharge of ampullary afferents as well as their response to rotation (Botta et al., 1998).

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Finally, betahistine administration has been shown to bilaterally reduce the immunoreactive staining for histamine in the posterior hypothalamus and in the vestibular nuclei, both in control conditions and following an unilateral labyrinthectomy (Tighilet and Lacour, 1996; Lacour, 1998), suggesting that it could increase the histamine turnover and synthesis in these brain regions (Lacour and Sterkers, 2001). Since all these actions of betahistine may potentially affect the transfer of labyrinthine information trough the vestibular nuclei, the therapeutic effects of this drug could be related, at least partially, to these mechanisms.

It is well known that the responses of both otolith organs and semicircular canals are critically dependent upon the direction of head movement (Wilson and Melvill Jones, 1979): most otolith units are maximally activated by head movements in a given ("preferred") direction and show null responses for movements in the perpendicular direction (Angelaki and Dickman, 2000). On the other hand ampullar units are maximally affected by rotation in the plane of the corresponding semicircular canal, while they do not respond to rotations in the plane perpendicular to the latter (Blanks et al., 1975). The temporal dynamics of vestibular afferents may vary considerably: the discharge of ampullar afferent is in phase with head velocity over a wide range of the physiological spectrum of head movements, while that of otolith afferents is mainly related to head position (Wilson and Melvill Jones, 1979). In general, the response phase of primary vestibular afferents is not dependent on the direction of head displacement (Angelaki and Dickman, 2000). Labyrinthine inputs with different spatial and temporal properties converge at the level of vestibular neurons (spatiotemporal convergence), giving rise to more complex response patterns: in fact, central neurons may show, in addition to the "preferred" direction of maximal response, a minimal response direction, perpendicular to the former (Angelaki, 1991, 1992; Angelaki et al., 1992; Bush et al., 1993). In some units the minimal response is close in amplitude to the maximal one, so that these neurons virtually lack a spatial specificity (Angelaki, 1991). All these units show response phases that vary according to the direction of stimulation (Angelaki, 1991). The aim of the present investigation was to study the effects of betahistine on the spatial and temporal response properties of secondary vestibular neurons located in the caudal, spinal-projecting region of the vestibular complex, in order to get insight into the mechanisms by which this drug may help the recovery of vestibular function following hemilabyrinthectomy.

2. Materials and methods

2.1. Animal preparation and unit recording

The experiments were performed in adult, urethane anesthetized (initial dose: 1.3 g/kg, i.p.), Winstar rats. All procedures complied with the National Institute of Health Guide for the Care and Use of Laboratory Animals, as well as with the European Communities guidelines for the use of experimental animals (Council Directive of 24/11/1986). In order to avoid pain and discomfort to the animal, the skin and the subcutaneous tissues incised during the surgery were infiltrated with novocaine; in addition, the levels of leg withdrawal and corneal reflexes, as well as electrocardiogram (recorded by needle electrodes) were continuously monitored during the experiment. The instantaneous heart frequency was evaluated by analyzing the electrocardiogram by a window discriminator and a rate meter. When the heart rate increased and/or corneal and leg withdrawal reflexes showed any tendency to recover, additional doses of the anaesthetic were administered.

Once that leg withdrawal and corneal reflexes were depressed by the anaesthesia, the spinous process of the T12–L1 spinal segments was exposed. The animal's head was fixed to a stereotaxic apparatus (pitched 20° nose-down), whose ear bars and head adapter (David Kopf) had been modified appropriately. In particular, coarse-tapered plastic caps substituted the ear bar tips, in order to avoid damage to the inner ear structures, while an aluminium plate that allowed distributing the pressure along the whole extent of the nasal bones replaced the nose-restraining bar of the head adapter. The animal body was secured to a spinal cord frame by a clamp placed on the T12–L1 spinous process. A rubber-heating pad prevented body displacement, while the limbs were taped. The animals were heated trough the body rubber pad, servo-controlled by a feedback system, so to maintain the rectal temperature between 37 and 38 °C.

Finally, small holes were drilled in the skull at coordinates appropriate for tracking in the vestibular nuclei (Paxinos and Watson, 1986). For unit recording and marking purposes, theta barrel glass microelectrodes were lowered through the cerebellum up to 4.5 mm below the surface. The recording barrel was filled with a 4M solution of NaCl, while the marking barrel contained Pontamine Sky Blue (5%). The micromanipulator was inclined by 25° with respect to the vertical, in order to avoid possible damage to the transverse sinus. Usually, the final point of each penetration in vestibular nuclei was marked by iontophoretic application of Pontamine (cathodal current, 30 μA, 15–30 min). A gel of Agar–Agar (2%) covered the exposed tissues to prevent drying.

2.2. Stimulation procedures and data analysis

The tilting table could rotate around three axes (transverse-pitch, longitudinal-roll and vertical-yaw) passing through the centre of the animal head. The roll and pitch axes of the table were driven with sinusoids out of phase of 90° (0.156 Hz, 5°). In this way, the animal was submitted to a "wobble "stimulus, i.e. to a tilt of constant amplitude (5°), whose direction rotated at constant velocity (56.2°/s) over the horizontal plane, either clockwise or counter clockwise (Schor et al., 1984). During clockwise stimuli, units recorded

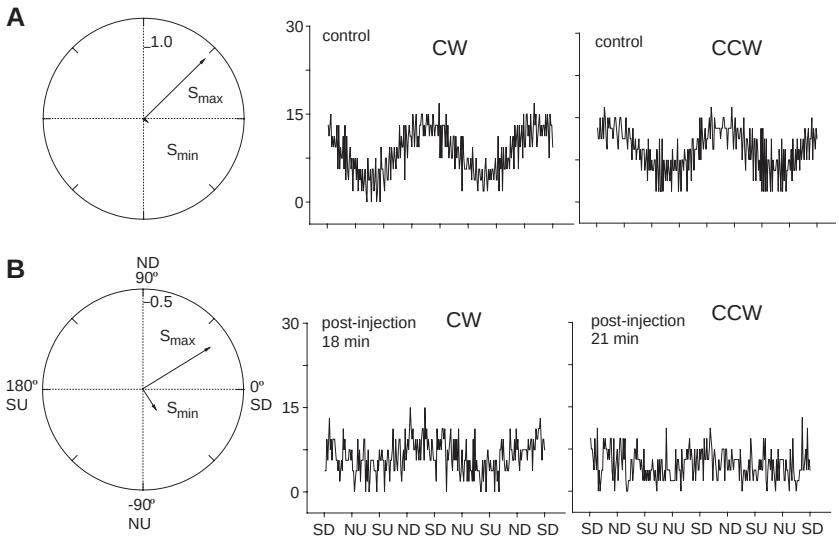

Fig. 1. Decreased responsiveness of a representative vestibular neuron to clockwise and counter clockwise wobble before (A) and after (B) i.p. injection of betahistine (150 mg/kg). The sequential pulse-density histograms on the centre and right columns have been recorded from a lateral vestibular nucleus neuron before (A, control) and after (B) drug delivery. They are the average of the activity recorded during 8 successive repetitions of two full periods of wobble. Gain and direction values obtained for the clockwise (CW) and counter clockwise (CCW) responses in A and B have been used for evaluating the response vectors shown in the corresponding polar diagrams as solid arrows. A. Before betahistine, the base frequency, gain and direction of the clockwise response corresponded to 11.6 impulses/s, 1.25 impulses/s/° and 43°, respectively. The corresponding values obtained for the counter clockwise response were 12.7 impulses/s, 1.07 impulses/s/° and 45°. The vector of maximal sensitivity (S_{max}), corresponding to the length of the solid arrow in the polar diagram, had a direction of 44° and a gain (G_{max}) of 1.16 impulses/s/°, while the vector of minimal sensitivity (S_{min}) was negligible, so that the unit could be classified as narrowly tuned (tuning ratio = 0.08). B. About 20 min following i.p. injection of a solution of betahistine (150 mg/kg), the base frequency and gain dropped respectively to 8.6 impulses/s and 0.59 impulses/s/° for the clockwise response and to 6.4 impulses/s and 0.33 impulses/s/° for the counter clockwise response. Due to these changes, the gain of the S_{max} vector decreased to 0.46 impulses/s/° and a significant S_{min} vector (G_{min} = 0.13 impulses/s/°) showed up. The tuning ratio value increased to 0.28, so that the unit was now broadly tuned. Following betahistine the direction of the clockwise and counter clockwise responses were to 31° and 33°, respectively, so that directions or the S_{max} and S_{min} vector corresponded to 33° and − 57°. SD: side-down, ND: nose-down, SU: side-up, NU: nose-up.

on the right side of the brain were successively analyzed during side-down, nose-up, side-up and nose-down animal tilt (see Fig. 1). During counter clockwise stimuli the sequence reversed to side-down, nose-down, side-up and nose-up. The cell firing frequency was fed to a rate meter and converted to standard pulses by a window discriminator. Occasionally, the original neuronal recordings could be analog-to-digital converted for illustration purposes. Fig. 2 shows two examples of single unit recordings, where extra cellular action potentials could be discriminated from the background noise. Criteria for considering a recording as originating from a single unit included the stability of spike

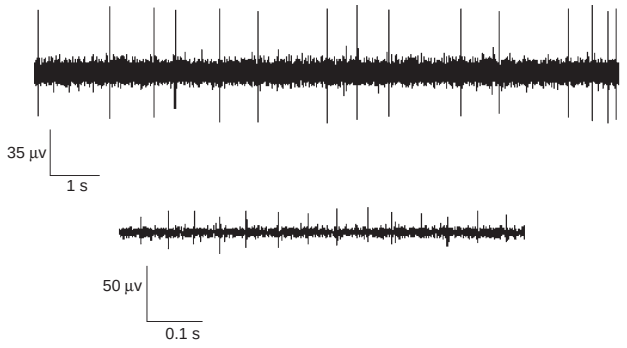

Fig. 2. Representative examples of extra cellular recordings from individual lateral vestibular nucleus neurons. In both instances the action potentials could be discriminated from the background activity.

shape and amplitude, as well as the lack of spike generation during the period of neuronal refractoriness, i.e. the absence of inter-spike intervals shorter than two ms. In order to control the duration of the inter-spike intervals, the sweep of the oscilloscope was continuously triggered by the output pulses of the window discriminator. The output of the window discriminator was also analysed by a signal processor that counted the number of spikes generated during 512 sequential intervals (bins) of 50 ms, which covered a stimulation sequence of four complete cycles of wobble in a given (clockwise or counter clockwise) direction (128 bins for each cycle). The instrument averaged the responses to 4–8 stimulation sequences, thus generating a four-cycle sequential pulse density histogram, which was converted, for illustration purpose, into a two-cycle display (see Figs. 1 and 3). These sequential pulse-density histograms were submitted to a fast Fourier analysis, which provided the base frequency (in impulses/s), i.e. the mean discharge rate of the unit during wobble, the gain (in impulses/s/°, indicated as G_{CW} and G_{CCW}, for clockwise and counter clockwise rotation, respectively) and the phase angle (in arc degrees) of the first harmonic component (0.156 Hz) of the averaged responses. The phase angle indicates the direction of head tilt giving rise to the peak discharge of the unit during wobble (response direction, D_{CW} and D_{CCW}): 0° and 180° corresponded to side-down and side-up displacements, while 90° and − 90° to nose-down and nose-up

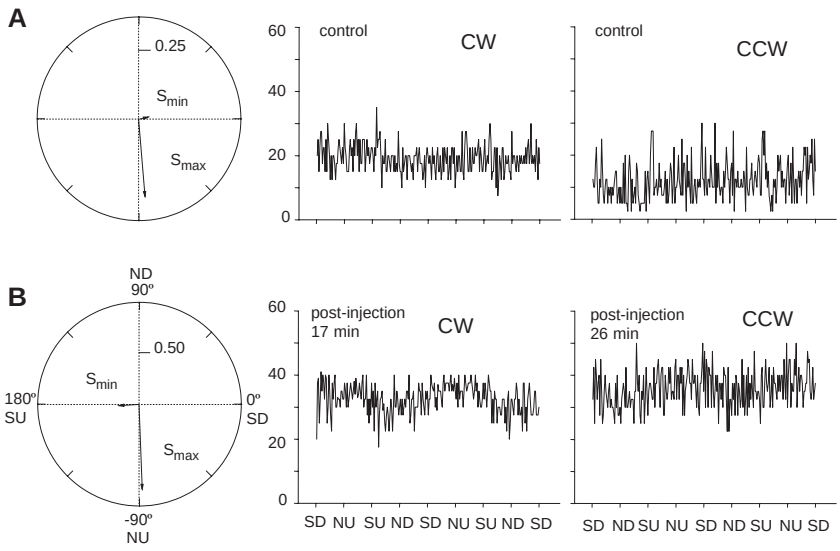

Fig. 3. Increased responsiveness of a vestibular neuron to clockwise and counter clockwise wobble before (A) and after (B) i.p. injection of betahistine (50 mg/kg). The sequential pulse-density histograms of the centre and right columns have been recorded by a descending vestibular nucleus neuron before (A) and after (B) drug delivery. They are the average of the activity recorded during 8 successive repetitions of two full periods of wobble. Gain and direction values obtained for the clockwise (CW) and counter clockwise (CCW) responses in A and B have been used for evaluating the response vectors shown in the corresponding polar diagrams as solid arrows. A. Before betahistine, the base frequency, gain and direction of the clockwise response corresponded to 19.2 impulses/s, 0.20 impulses/s/° and −126° respectively, while the corresponding values obtained for counter clockwise responses were 12.2 impulses/s, 0.26 impulses/s/° and −45°. However, in both instances, signal-to-noise ratio and coherence values were sub threshold for responsiveness, being lower than 0.5. The S_{max} vector shown in the polar diagram (G_{max}=0.23 impulses/s/°, θ_{max}=− 85°) is therefore insignificant. B. Following betahistine, base frequency, gain, signal-to-noise ratio and coherence of both clockwise and counter clockwise responses increased, and a modulation of unit activity became evident. The response parameters of the clockwise response changed to: base frequency=34.4 impulses/s, gain=0.85 impulses/s/°, signal-to-noise=0.83, coherence=0.85. For the counter clockwise response we had: base frequency=35.7 impulses/s, gain=0.51 impulses/s/°, signal-to-noise=0.84, coherence=0.84). The directions of the clockwise and counter clockwise responses corresponded to − 100° and − 77°, respectively. While the vector of maximal sensitivity (S_{max}, see polar diagram) increased in gain (G_{max}=0.67 impulses/s/°) with respect to control, its direction (θ_{max}=− 88°) was close to the pre-injection value. It is of interest that, following betahistine, the unit was broadly tuned, with a gain (G_{min}) of the minimal sensitivity vector (S_{min}) of 0.17 impulses/s/° and a tuning ratio of 0.25. SD: side-down, ND: nose-down, SU: side-up, NU: nose-up.

displacements, respectively (see Figs. 1 and 3, polar diagrams). Side-down and side-up positions were referred to the side of unit recording.

The base frequency value closely corresponded to the unit discharge obtained at rest. In order to assess the unit responsiveness, the coherence and the signal-to-noise ratio were calculated. The coherence corresponded to the square amplitude of the ratio between the vectorial and the algebraic mean of the response amplitudes (in impulses/s) relative to the four wobble cycles that formed the stimulation sequence. The value 1 was obtained only when these four gain and phase values were equal. The signal-to-noise ratio corresponded to the ratio between the amplitude of the fundamental harmonic of the response to the root-mean-square amplitude of all harmonics above the second. We considered responsive those records with both coherence and signal-to-noise ratio > 0.5. Occasionally, signal-to-noise ratio values included between 0.4 and 0.5 could be also accepted, provided that the unit response parameters did not change by repeating the test.

2.3. Evaluation of the response vectors

The response (sensitivity) vectors of a given cell are characterized by gain (G), direction (θ) and temporal phase (φ) components (Schor and Angelaki, 1992), which can be estimated by the responses to both clockwise and counter clockwise stimuli (Angelaki, 1992; Schor et al., 1984) (see Figs. 1 and 3). In the present experiments, the temporal phase was evaluated with respect to animal position. Negative and positive values of φ represented respectively lags and leads of the peak of unit activity with respect to the peak of animal displacement.

The modulation in firing rate during a sinusoidal tilt in a given direction can be predicted by summating the projections of the cell response vectors along the stimulus direction. The projection of each vector corresponds to: G cos α sin ($\omega t+\varphi$), where α is the angle included between θ and the direction of tilt (Schor and Angelaki, 1992). Neurons affected by both clockwise and counter clockwise rotations (*bidirectional units*) are directionally tuned. They can be described by one vector of maximal (S_{max}) and one of minimal (S_{min}) sensitivity (Angelaki et al., 1992, 1993; Schor and Angelaki, 1992; Bush et al., 1993), spatially and temporally orthogonal. For these neurons:[1] $G_{max}=(G_{CW}+G_{CCW})/2$; $G_{min}=|G_{CW}-G_{CCW}|/2$; $\theta_{max}=(D_{CW}+D_{CCW})/2$;

[1] All the following computations are relative to units recorded on the right side. For left side recorded units CW and CCW parameters have to be reciprocally exchanged.

$\theta_{min}=\theta_{max}-90°$ (for $G_{CW}\geq G_{CCW}$), $\theta_{min}=\theta_{max}+90°$ (for $G_{CW}<G_{CCW}$); $\varphi_{max}=(D_{CW}-D_{CCW})/2$; $\varphi_{min}=\varphi_{max}+90°$. "Narrowly tuned" neurons show similar response gains to clockwise and counter clockwise wobble, display a maximal response for stimuli oriented in the S_{max} direction and a null response for stimuli in the perpendicular direction (i.e. S_{min} is null). "Broadly tuned" units show different response amplitudes to clockwise and counter clockwise wobble and, in addition to the maximal response, display also a minimal, non-zero response for stimuli in the direction of S_{min}. A broadly tuned behaviour indicates that signals with different spatial and temporal characteristics converge at unitary level (Schor et al., 1984; Baker et al., 1985; Angelaki et al., 1992). This process of spatiotemporal convergence can be quantified by the "tuning ratio"(G_{min}/G_{max}), which is related to the ratio G_{CW}/G_{CCW} (Angelaki, 1992). Units are considered as broadly tuned when their tuning ratio is higher than 0.1. Broadly tuned neurons showing tuning ratio equal to 1.0 respond only to wobble in one direction (either clockwise or counter clockwise) and display the same sensitivity, irrespectively upon the stimulus direction in the horizontal plane (unidirectional units).

2.4. Experimental procedure

The experiments started with a systematic tracking aimed to localize the region of vestibular nuclei, identified on the basis of the presence of units responsive to wobble. This recording period of control responses preceded the drug injection and could last up to 4 h. Finally, a stable unit with a well-isolated spike was tested for betahistine effects. The drug, dissolved in saline, at the concentration of 50 mg/ml, was injected intraperitoneally. Usually the injected dose corresponded to 50 mg/kg. In three experiments a dose of 150 mg/kg was utilized. Once betahistine was delivered, the neuronal responses to wobble were recorded at regular intervals until the unit could be discriminated from background noise. When the unit was lost, the recording of neuronal activity continued in the same and adjacent tracks up to 4 h following betahisthine administration. No further injections of the drug were performed.

2.5. Statistical analysis

In order to verify whether the recorded units could be grouped in separated populations (clusters), we performed a hierarchical cluster analysis (Blashfield, 1980). This algorithm, belonging to a SPSS statistical package, grouped the data in order to minimize the "distance" between all possible pairs of points formed between clusters (procedure of linkage between-groups). The "distance", was measured in a three-dimensional space whose axes corresponded to the gain of maximal sensitivity vector, the base frequency and the ratio of the two variables. In grouping the units, no a priori assumption was made about the number of clusters included within the recorded population. Statistical comparison between control and post-injection data were performed by analysis of variance (ANOVA). The significance level was set at $P<0.05$.

3. Results

3.1. Recorded population

Only a limited population of cells ($n=8$) could be tested before and after betahistine administration. These neurons were located within the lateral vestibular nucleus ($n=3$) and the descending vestibular nucleus ($n=5$). For 5 of these units (2 in the lateral vestibular and 3 in the descending vestibular nucleus) a dose of 50 mg/kg of betahistine was utilized, while for the remaining three units the dose was 150 mg/kg. Seven of the units tested before and after the drug were responsive to both clockwise and counter clockwise stimuli, while one was unsensitive (see Fig 3A). The evaluation of the time course of the effects of betahistine on single units and a comparison between the data recorded in the control period and those relative to the post-injection period were performed. In particular, 108 neurons (81 in the lateral vestibular nucleus, 27 in the descending vestibular nucleus) were recorded before and 34 (17 in the lateral vestibular and 17 in the descending vestibular nucleus) after betahistine delivery at the dose of 50 mg/kg.

3.2. Effects of betahistine on single neurons

A clear effect of betahistine on neural responses to labyrinthine stimulation could be observed in all the eight units analyzed before and after drug delivery. On the other hand, in two units, injection of an equal volume of saline did not modify either the gain or the response direction to clockwise and counter clockwise wobble.

In all the three units (2 in the lateral vestibular and 1 in the descending vestibular nucleus) tested before and after the highest dose (150 mg/kg) of betahistine a clear post-injection decrease in the gain of the maximal sensitivity vector (G_{max}) could be observed. On the average, the gain dropped to 79%, 51% and 47% of the control value at 15, 30 and 45 min following betahistine (ANOVA, $P<0.005$), the corresponding base frequency values being 117%, 89% and 83%, respectively (ANOVA, N.S.). The relative stability of this parameter was due to the fact that two units showed a decrease in base frequency, while the third one was characterized by a 50% increase. The average changes observed in the remaining response parameters (θ_{max}, φ_{max}, G_{min}, tuning ratio) were not significant. One of these units is illustrated in Fig. 1. In this instance, before the injection, the responses to clockwise and counter clockwise wobble showed gains of 1.25 and 1.07 impulses/s/°, respectively, that corresponded to a G_{max} value of 1.16 impulses/s/°. About 20 min following betahistine delivery, G_{CW} and

G_{CCW} dropped respectively to 0.59 and 0.33 impulses/s/°, corresponding to a G_{max} value of 0.46 impulses/s/°. In the control condition this unit was narrowly tuned (tuning ratio = 0.08), with a G_{min} value of 0.09 impulses/s/°. Following betahistine, G_{min} increased to 0.13 impulses/s/°, as indicated by the gain difference observed between the clockwise and the counter clockwise response. The unit became therefore broadly tuned (tuning ratio = 0.28), thus being more sensitive to stimuli oriented perpendicularly to the preferred direction. The direction of the S_{max} vector of the unit rotated clockwise by 12° following betahistine (from 44° to 33°, see polar diagrams) while its phase was always close to 0° (not shown).

When the lowest dose of betahistine (50 mg/kg) was injected, a depression in both base frequency and G_{max} was observed in three units (2 in the lateral vestibular and in 1 the descending vestibular nucleus). In particular, G $_{max}$ dropped progressively to 77%, 44% and 27% of the control value at 15, 30 and 45 min following drug delivery (ANOVA $P<0.05$). The corresponding base frequency values were 87%, 59% and 42% (ANOVA $P<0.05$). On the average, no significant changes in φ_{max}, θ_{max}, G_{min} and tuning ratio could be observed.

The remaining two units studied before and after the lowest dose of betahistine (50 mg/kg) showed a prominent gain increase following drug delivery. In particular, the representative unit shown in Fig. 3 was characterized by a substantial lack of modulation in the control condition where both clockwise and counter clockwise responses showed coherence and signal-to-noise ratio values sub threshold for responsiveness (see Fig. 3A). A clear activity modulation during wobble became apparent about 15 min following betahistine (Fig. 3B). An increase in base frequency was simultaneously observed. In particular, at this time the neuron showed a clockwise response characterized by a gain of 0.85 impulses/s/° and a direction of 100°. The corresponding values obtained for the counter clockwise response were 0.51 impulses/s/° and 77°, respectively. The neuron was therefore broadly tuned with G_{max} and G_{min} values of 0.68 and 0.17 impulses/s/°, respectively (tuning ratio = 0.25). The direction of the maximal sensitivity vector (θ_{max}) was − 88° with a phase (φ_{max}) of about 12°. It is of interest that the θ_{max} post-injection value was close to that evaluated in control condition, by Fourier analysis of the sub threshold responses of Fig. 3A. The second unit that showed an increase in G_{max} following the lowest dose of betahistine was clearly responsive before drug delivery and showed a substantial stability of the remaining response parameters as well as of the base frequency.

3.3. Comparison of "control" and "post-injection" populations

When all the data recorded before betahistine (50 mg/kg) were compared with those recorded in the post-injection period, it appeared that 57/108 neurons in the former and 20/34 neurons in the latter population, were affected by wobble. In the control condition 41 neurons were affected by both clockwise and counter clockwise wobble (bidirectional units), and 16 by stimuli running only in a given direction, either clockwise or counter clockwise (unidirectional units). Following betahistine, 17/20 units were bidirectional and 3/20 unidirectional. The distribution of unresponsive, unidirectional and bidirectional units did not differ significantly between control and post-injection population (chi square). Moreover, no significant differences could be found in the average base frequency values, between the control (18.2 ± 14.2, S.D., impulses/s) and post-injection group (22.7 ± 15.5, S.D., impulses/s) (ANOVA N.S.). This result was obtained for both responsive and unresponsive units as well as for the whole population. When bidirectional units were taken into account, no significant difference could be found between the average control and post-injection values of the gain of the maximal sensitivity vector (G_{max}), which corresponded to 0.66 ± 0.60, S.D., impulses/s/° and 0.52 ± 0.25, S.D., impulses/s/°, respectively. However the two groups of responses differed in their variability, as indicated by the S.D. values (Fischer, $P<0.0005$). Fig. 4 illustrates how this effect could arise. As shown in Fig. 4A there was a positive correlation between G_{max} and base frequency values. The visual inspection of the scatter diagram suggested that the units could be grouped in two populations characterized by a different ratio between the two parameters. Indeed, a hierarchical cluster analysis based on G_{max}, base frequency and the ratio of the two variables, confirmed this assumption, indicating that two distinct clusters could be identified. A first population of high-gain units (average G_{max} value. 1.67 ± 0.47, S.D., impulses/s/°, $n=10$) showed a steep relation between G_{max} and base frequency values (regression line: $y=0.0361x+0.847$, $r=0.723$, $P<0.02$). A second population of low-gain units (average G_{max} value: 0.36 ± .20, S.D., impulses/s/°, $n=31$) was characterized by a smaller increase in G_{max} for a given increase in basal activity (regression line: $y=0.0076x+0.207$, $r=0.63$, $P<0.001$). The difference in the average G_{max} value between the two groups was highly significant (ANOVA $P<0.001$). Neurons located in the lateral vestibular (Fig. 4, dots) and in the descending vestibular nucleus (Fig. 4, circles) could be observed in both populations. On the other hand, following betahistine, the recorded population was rather homogeneous (see Fig. 4B), with a regression line of the relation between G_{max} and base frequency corresponding to $y=0.010x+0.32$ ($r=0.72$, $P<0.001$). The average post-injection value of G_{max} was significantly different from those of both populations of high- and low-gain neurons recorded in the control condition ($P<0.001$ and $P<0.02$ for comparison with high- and low-gain units, respectively).

While the largest G_{max} values disappeared following the injection, the behaviour of G_{min} was quite different. A

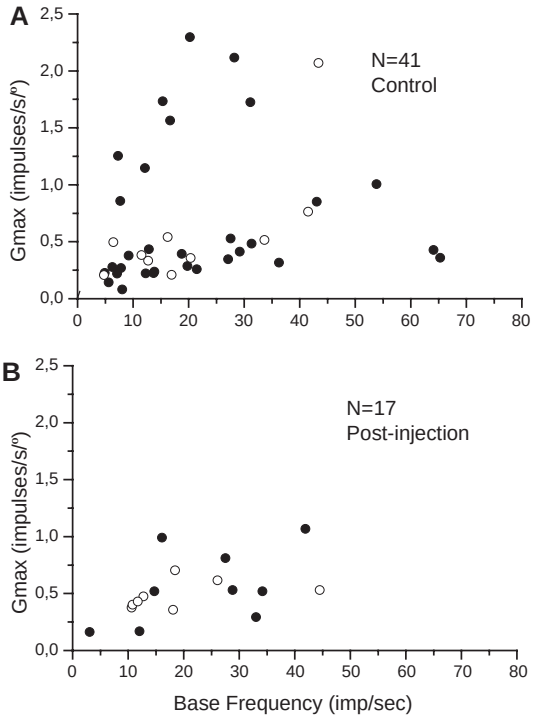

Fig. 4. Relation observed between the gain (G_{max}) of the maximal sensitivity vector and base frequency values before (A) and after (B) i.p. injection of betahistine (50 mg/kg). A. Data obtained before drug delivery. The scatter plot of G_{max} and base frequency values revealed two populations of high-gain and low-gain units, characterized by different regression lines of the relation between the two parameters (see text). B. Data obtained following betahistine, from 5 to 240 min after drug delivery. The scatter plot indicated the presence of only one population of neurons. Both in A and B filled and unfilled circles represent lateral vestibular and descending vestibular neurons, respectively.

significant difference existed between the average control (0.07±0.06, S.D., impulses/s/°) and post-injection values (0.12±0.13, S.D., impulses/s/°) of this parameter (ANOVA $P<0.05$). The same trend was observed for both lateral vestibular and descending vestibular neurons. Scatter plots of G_{min} versus base frequency indicated that there was no clustering of the units in different sub-populations, both before and after betahistine. As a consequence of the changes in G_{max} and G_{min}, drug delivery induced a significant change (ANOVA $P<0.02$) in the average tuning ratio value, which increased from 0.12±0.02, S.D. to 0.20±0.16, S.D. As shown in Fig. 5, about 46% of bidirectional units (19/41) could be classified as narrowly tuned in control condition (tuning ratio<0.1, see Fig. 5A), while the proportion was reduced to 18% (3/17) following drug delivery (Fig. 5B). Similar results were obtained for both lateral vestibular and descending vestibular neurons.

In spite of the modifications in G_{max}, G_{min} and tuning ratio, no major changes could be observed in the distributions of θ_{max} and θ_{min} following betahistine. However the average φ_{max} value was significantly modified within the lateral vestibular nucleus, where it increased from an average of 6.1±37.9, S.D., ° ($n=31$) to 40.6±18.5, S.D., ° ($n=9$), the difference being statistically significant (ANOVA $P<0.02$).

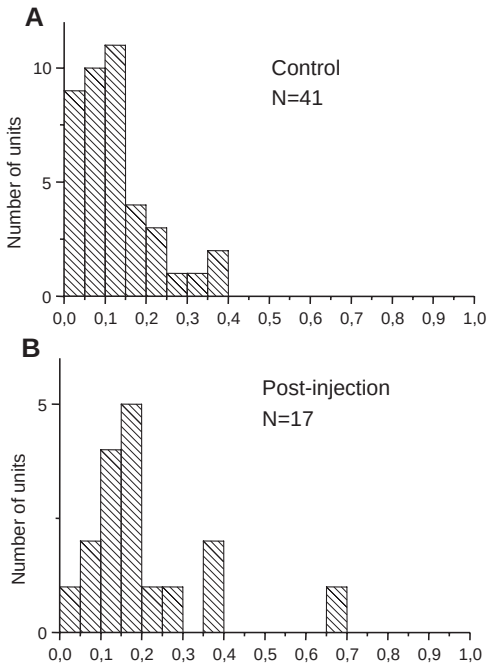

Fig. 5. Distribution of tuning ratio values observed before (A) and after (B) i.p. injection of betahistine (50 mg/kg). A. Before betahistine administration 46% of the units (19/41) showed tuning ratio values < 0.1, being therefore classified as narrowly tuned. B. Following betahistine, the proportion of narrowly tuned units decreased to 18% (3/17).

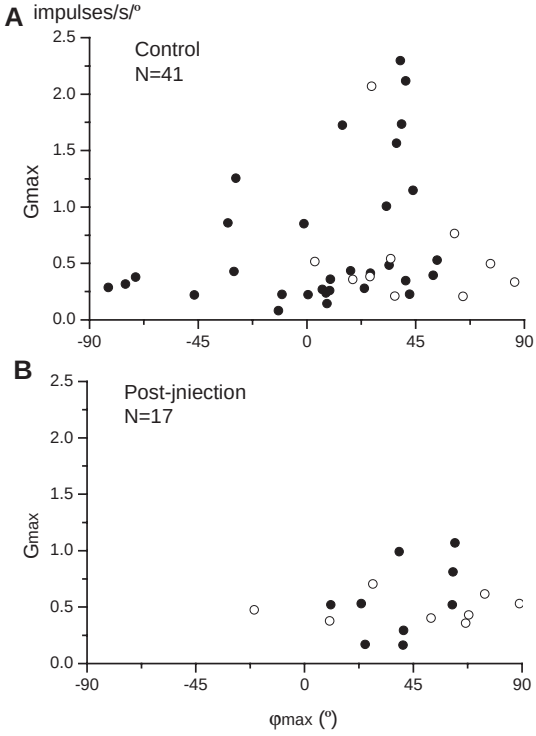

Fig. 6. Scatter plot of gain (G_{max}) and phase (φ_{max}) value of the maximal sensitivity vector (S_{max}) obtained before (A) and after (B) i.p. injection betahistine (50 mg/kg). Positive and negative values in abscissa represent phase leads and lags, respectively. Filled and unfilled circles represent lateral vestibular nucleus and descending vestibular nucleus neurons, respectively.

A scatter plot of G_{max} versus φ_{max} indicated that high gain units with $G_{max} > 1.0$ impulses/s/° and φ_{max} included between − 45° and 45° could not be recorded following betahistine (see Fig. 6). However, no φ_{max} modifications were observed following betahistine within the descending vestibular nucleus.

4. Discussion

4.1. General considerations

It has to be pointed out that, in our experiments, the exact site of action of betahistine cannot be determined with certainty, given the i.p. route of drug delivery. Betahistine interacts with histamine H_1 and/or H_3 receptors localized in the whole brain (Schwartz et al., 1990; Schwartz, 1997; Bouthenet et al., 1988;), including the vestibular complex (Yabe et al., 1993; Wang and Dutia, 1995; Pillot et al., 2002), and modifies the blood perfusion of the vestibular apparatus (Dziadziola et al., 1999): these changes may induce modulation in the release of transmitter from receptors to afferents, thus leading to changes in their activity (Park et al., 2004) and, possibly, in the vestibular response of central neurons. So, multiple mechanisms of actions could account for the observed effects. It is unlikely, however, that the observed effects are solely attributable to the hypotension that can be induced by the drug (Laurikainen et al., 1993) and which would lead to an increase in the heart rate (Heymans, 1929). In fact, while the increase in cardiac frequency induced by an i.p. injection of betahistine (50 mg/kg) reached its peak 15 min following drug delivery and then dropped progressively towards the control level, betahistine effect on neuronal activity were still fully developed at 45 min from the injection.

4.2. Nature of the observed effects

The effects of betahistine on neuronal activity and labyrinthine responses of vestibular neurons observed in the present experiments were complex. When the units tested both before and after betahistine were taken into account, enhancements, depressions or no effects on the base frequency could be observed. According to this finding, the base frequency of all the neurons recorded *before* drug delivery was not significantly different from that of units recorded *afterwards*.

It is of interest that a depression of the spontaneous activity of lateral vestibular neurons, polysynaptically (but not monosynaptically) activated by the labyrinth, has been observed, in vivo, following i.v. or microiontophoretic administration of betahistine (Unemoto et al., 1982), at doses lower than those utilized in the present experiments. This effect could be expected on the basis of the fact that the drug depresses the firing rate of ampullar afferents (Botta et al., 1998). So far, excitatory effects of betahistine on the spontaneous activity of vestibular neurons have been shown following local administration of the drug in slices (Wang and Dutia, 1995), where the action of H_3 histamine receptor, a drug target, is not present.

While at the highest dose of betahistine the gain of the maximal sensitivity vector was invariably decreased, at single unit level, the changes in responsiveness elicited at the lowest concentration could be of opposite sign. According to this observation, analysis of the scatter plots of G_{max} and base frequency, obtained for the whole recorded population, suggests that high gain responses were depressed, while low-gain responses were enhanced by the drug. Moreover, the minimal response gain of the units was increased, indicating that the responsiveness of the units to the weakest inputs tended to be enhanced by betahistine.

These gain modifications could be only partially attributed to changes in neuronal excitability. In fact, modifications in G_{max} and G_{min} could occur without base frequency changes, or could show opposite sign with respect to the latter. The finding that a given unit may show a decreased responsiveness to the strongest labyrinthine input, while the response to the weakest input is not affected or enhanced by betahistine suggests that presynaptic mechanisms could be involved in the effects of the drug. From this point of view it is of particular interest that H_3 presynaptic receptors, that represent a high-affinity binding-site for betahistine, can be expressed also on non-histaminergic afferents (Lacour, 1998). So far no evidence had been given that neuronal responsiveness to natural vestibular stimulation could be enhanced by betahistine. In fact, the bath applied drug induced a depression in the responses of ampullar afferents to mechanical stimulation (Botta et al., 1998), while a stronger, depressive action was exerted by microiontophoretic administration of betahistine on the responses of medial vestibular nucleus type I neurons to horizontal rotation (Kawabata et al., 1991).

4.3. Pharmacological aspects

The high dose of betahistine used in the present experiments is comparable to that used by Lacour and collaborators (Tighilet et al., 1995), who have shown that daily administration of 50–100 mg/kg of the drug may speed up the process of vestibular compensation. As pointed out by these authors, such high doses of betahistine increase histamine turnover (synthesis) and release by blocking H_3 autoreceptors (see also Lacour and Sterkers, 2001). An increased level of this monoamine within the vestibular nuclei could potentially reduce the unbalance in the activity of the corresponding neurons between the two sides, thus leading to a faster compensation (Lacour and Sterkers, 2001). Moreover an increased release of monoamines enhances alertness and vigilance (Lin, 1994), which could play a role in vestibular compensation, as indicated by the fact that stimulants speed up, while sedatives depress this process (Peppard, 1986).

The betahistine effects observed in the present experiments could contribute to the compensation process by improving the compensation of "dynamic" vestibulospinal symptoms (Smith and Curthoys, 1989). Unilateral lesions of the peripheral vestibular system induce a decrease in the activity of ipsilateral limb extensor muscles. This can be attributed to the depression, induced by the lesion, in the activity of the lateral vestibular neurons, that exert a facilitatory action on ipsilateral limb extensor motoneurons. While forelimb responses to vestibular stimulation are hardly elicited on the lesion side, due to the lack of muscle tone, they are present, but with a reversed pattern, on the "intact" side (Berthoz and Anderson, 1972). In a decerebrate cat with both VIII nerves intact, side-down roll tilt of the whole animal increases the ipsilateral forelimb extensor tone while side-up tilt induces the opposite effect. In fact, lateral vestibular neurons are mainly excited during side-down, while depressed during side-up animal tilt. Following unilateral VIII nerve neurectomy, the vestibular responses of lateral vestibular nucleus neurons localized on the "intact" side show a reversal of the predominant pattern. In this condition, most of the neurons are excited during side-up, rather than side-down animal tilt (Lacour et al., 1985). We have shown that betahistine depresses the gain of the largest responses to animal rotation, while enhancing weak or sub-threshold vestibular inputs. It may well be that, in hemilabyrinthectomized animals, this action of betahistine leads to a decrease of the predominant, "wrong" pattern of response to the labyrinthine input of lateral vestibular nucleus neurons and helps to re-establish the normal response pattern. The postulated effect could help the recovery of vestibulospinal reflexes, thus speeding up the process of compensation.

As already mentioned, the classical therapeutic effects of betahistine are obtained at doses much lower than those utilized in the present experiments (Lacour and Sterkers, 2001; Mira, 2001). However, at least one of the betahistine effects observed in the present experiments could be involved with this action. We have in fact shown that betahistine decreases the spatial selectivity of vestibular neurons. This implies that a smaller modulation of neuronal discharge would occur in vestibular nuclei during the changes in the direction of head movement that may take place during normal motor behaviour. It is possible that this effect may contribute to reduce peripheral vertigo, which could be associated to an abnormal process of labyrinthine signals. Further studies are necessary in order to document whether this effect persists even at the doses of the drug that are effective in the treatment of labyrinthine disorders.

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